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# Orientation and flight behaviour identify the Soprano pipistrelle as a migratory bat species at the Baltic Sea coast

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Short title: Migratory orientation of pipistrelle bats

**Abstract**

Migration routes of bats remain largely unknown, as previous orientation studies have been challenging even with newly developed techniques in tracking, genetic and stable isotope studies. However, a lack of knowledge about migrations poses problems for species conservation, especially in newly described species for which ecological information is not yet available. Here, we aimed to test flight orientation behaviour in the Soprano pipistrelle, *Pipistrellus pygmaeus*. This species has been described only 22 years ago but is now known to have a wide geographic distribution in Europe, yet virtually no information exists about seasonal movements of *P. pygmaeus*. In large parts of the continent, seasonal occurrence of *P. pygmaeus* matches with that of long-distance migratory Nathusius' bats (*P. nathusii*). To shed light on the migratory behaviour of both species, we investigated their orientation decisions at the Latvian Baltic Sea coast which is well-known for summer bat migration along a north-south axis. We developed an arena-based assay designed to measure orientation of takeoffs. The arena was installed in the natural flight path of *Pipistrellus nathusii* and *P. pygmaeus*, and after takeoff, bats chose the direction freely. We detected bearing fidelity between takeoff and departure flights, suggesting bats used cues within the arena, putatively geomagnetic information, which allowed them to set a course prior to takeoff. Further, our results show *P. pygmaeus* orientates in a southerly, seasonally appropriate direction, similar to *P. nathusii* during on-going migration. Therefore, our findings are consistent with true migratory behaviour of *P. pygmaeus* in the northern part of its range. Predicting flight directions of bats based on takeoff direction offers a simple test for orientation studies, and could further be used to test senses of bats under varying treatments, thereby facilitating a comparison of navigational skills across taxa, e.g. bats and birds.

**Keywords**: Animal orientation, Bat migration, Mammal migration, Movement Ecology, Navigation, *Pipistrellus nathusii*, Take-off

## Introduction

Bats living in seasonal habitats migrate to reach mating and wintering grounds, yet orientation behaviour and spatial movements of bats remain poorly described for most species owing to the cryptic nature of bat migration (Holland 2007; Voigt et al. 2018). The traditional method of ringing bats with metallic bands uncovered some of the longest continental mammal migrations in the world (Barclay & Bell 1988; Hutterer et al. 2005). While ringing has been shown to be an effective solution for the study of spatial movements, it needs to be applied to vast numbers of bats to ensure reliable data because of often low recovery rates (Barclay & Bell 1988; Steffens et al. 2004; Ellison 2008; Holland & Wikelski 2009). Also, some species are sensitive to ringing which led to a reduction of ringing efforts based on ethical reasons (Baker et al. 2001; Dietz et al. 2006). Due to these limitations, our understanding of regional bat movements and migration has been largely restricted to selected species and study locations harbouring stable populations.

However, migratory behaviour has been newly described or revisited for many bat species over the past decades based on traditional ringing and rapid developments of other techniques in both field and laboratory (Wilkinson & Fleming 1996; Russell et al. 2005; Morales-Garza et al. 2007; Racey et al. 2007; Bryja et al. 2009; Moussy et al. 2013). For example, genetic techniques have revealed migratory directions could be inferred from pairwise comparisons of haplotype frequencies of common noctules, *Nyctalus noctula*, sampled in nursery roosts and hibernaculas (Petit & Mayer 2000). However, both dispersal and migration affect gene flow which makes exact delineations of migratory behaviours based on genetic patterns vulnerable to subadult dispersal movements (Moussy et al. 2013). Further, mating on migration, a common behavioural strategy observed in

European and North American bats, e.g. *Tadarida brasiliensis*, can hamper assignments of individuals to geographically distinct subpopulations (McCracken & Gassel 1997).

Alongside this, automated acoustic recordings of bat echolocation calls represent an alternative technique to detect spatiotemporal occurrences of bats, often suggestive of migratory movements (Heim et al. 2016). Such acoustic monitoring is highly valuable for delineating the phenology of mass occurrences of bats at distinct locations and enable us to identify putatively migratory species by call characteristics (Jarzembowski 2003; Furmankiewicz & Kucharska 2009; Johnson et al. 2011; Rydell et al. 2014). In combination with visual observations, such acoustic monitoring might even enable determination of the direction from which bats arrive at specific locations (Furmankiewicz & Kucharska 2009). Yet, acoustic monitoring alone does not reveal the direction of migratory movements of individual bats, and therefore the observation of seasonal presence of bats using acoustic methods fails to provide convincing evidence for migration

Lastly, stable isotope approaches have revealed the geographical origins of sedentary and migratory bats based on isoscape origin models (Cryan et al. 2004; Ossa et al. 2012; Popa-Lisseanu et al. 2012; Voigt et al. 2012; Lehnert et al. 2014; Voigt et al. 2016), yet the accuracy of these geographic assignments suffers from variability of isotope ratios in consumer tissues and source waters (Voigt & Lehnert 2018). Finally, our understanding of regional and long-distance migration is hampered in bats because conventional approaches are largely incompatible with their small size, which presents tagging bats with long-lasting loggers or GPS units (Holland & Wikelski 2009).

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Yet , we recently used the direction of departure flights of bats tagged with radio transmitters to infer migratory orientation (*Pipistrellus nathusii*; Lindecke et al. 2015). These bats showed a

consistent southern departure flight direction even when translocated 11 km far away from the migration corridor where they were captured (Lindecke et al. 2015). Based on that, we aimed to develop a method to study bat orientation and eventually migration. Here, we investigated the migration behaviour of a cryptic bat species, the Soprano pipistrelle, *Pipistrellus pygmaeus* (Leach, 1825). Soprano pipistrelles are particularly interesting with respect to their movement ecology, because this species was once considered to be identical to *P. pipistrellus* (Schreber, 1774), a sister species which lacks any pronounced migration behaviour (Steffens et al. 2004, Hutterer et al. 2005). At the turn of the last century, *P. pygmaeus* was described as a new species based on genetic, morphological and acoustic parameters (Barratt et al. 1997; Mayer & von Helversen 2001). Therefore, banding data preceding this date accidentally lumps recapture data of the two species, confounding our understanding of the movement ecology of both species. Recent field studies demonstrated both species prefer distinct habitats, exhibit contrasting foraging behaviours and show different phenologies in large parts of their distribution range (Davidson-Watts & Jones 2006; Davidson-Watts et al. 2006; Nicholls & Racey 2006; Sattler et al. 2007). While *P. pipistrellus* is widely accepted as a sedentary, or eventually regionally migratory bat (Hutterer et al. 2005; Voigt et al. 2012; Voigt et al. 2016; but cf. Bryja et al. 2009), the status of *P. pygmaeus* as a potentially migratory species is still under debate, albeit in-depth genetic studies implied some degree of migratory behaviour for this species (Racey et al. 2007; Bryja et al. 2009; Dietz et al. 2009). Further genetic analysis suggested that migratory behaviour of *P. pygmaeus* is more pronounced in continental European populations than in populations of the British Isles (Sztencel-Jablonka & Bogdanowicz 2011).

takeoffHere, we focused on the orientation behaviour of individual *P. pygmaeus* caught between August and September at Pape Bird Ringing Station (PBRS) at the Latvian Baltic Sea coast. Previous acoustic studies demonstrated a regular occurrence of the species at PBRS during summer

migration (Rydell et al. 2014; Voigt et al. 2017, 2018), yet it is unknown if this reflects the seasonal occurrence of *P. pygmaeus* in this region without pronounced directional migration flights. Interestingly, similar to other bats passing PBRS on migration, *P. pygmaeus* only exceptionally displays foraging behaviour (Voigt et al. 2017, 2018). In our study, we aimed to compare directionality of flights, here estimated as the orientation behaviour of departing *P. pygmaeus* with that of a well-known European long-distance migrant, *P. nathusii*. Nathusius' bats have been extensively studied at the same location for many years (Pētersons 2004; Steffens et al. 2004, Hutterer et al. 2005) and show pronounced southward orientation when released close to the coastal line of PBRS (Lindecke et al. 2015).

In order to investigate the migratory behaviour of *P. pygmaeus* and *P. nathusii* without the use of tracking devices, we aimed to first identify a movement behaviour which is performed by all bats and which is measurable at a relatively small scale, yet with sufficient accuracy and precision. In the past, numerous attempts to measure orientation behaviour of bats in a confined space failed (e.g. Mueller 1966), however Wang et al. (2007) demonstrated that Chinese noctules (*Nyctalus plancyi)* aligned themselves to the magnetic field when resting in a round plastic basket. In studies of non-migratory bats, Holland and colleagues also demonstrated that bats use a magnetic compass during active flight (Holland et al. 2006; Holland et al. 2010). Based on these studies, we hypothesized that wild bats could use environmental cues, putatively the Earth's magnetic field, for orientation and also directional takeoff from the perimeter of a circular arena. Thus, we predicted that if a bat oriented towards a relatively distant (migratory) goal from inside a circular-shaped test apparatus, we would find bearing fidelity between measures. That means, we would observe persistence between repeated measures of direction, i.e. maintenance of a compass bearing while the focal bat is moving freely. In theory, high bearing fidelity indicates high relevance of the particular movement direction to the individuals' navigational decisions, sometimes despite

elevated sensory noise or dynamically changing environmental information. To test our prediction on bearing fidelity in pipistrelles, we developed a simple, easy-to-build setup which we termed a *circular release box* (CRBox) for bats enabling us to register a bats' takeoff orientation (TOO). We observed the directionality of flights, i.e., initial departure flight orientation (DFO) of free flying bats after takeoff from the CRBox. In bird orientation studies, recording of initial movement bearings is standard (Wallraff, 2005), and it has been successfully used in hand-released bats in the past (e.g. Mueller 1966; Buchler & Childs 1982; Serra-Cobo et al. 2000). If *P. pygmaeus* oriented departure flights easterly towards inland instead of the Baltic Sea shoreline, i.e. in stark contrast to *P. nathusii,* we would reconsider our hypothesis of a true migratory motivation in *P. pygmaeus*.

Aside from spatial orientation measures, we also timed latency to takeoff. Latency, the delay between a stimulus and a behavioural response, is a standard measure in behavioural assays, e.g., in studies of spatial learning and escape responses (Sousa et al., 2006; Domenici et al., 2011). Conceptual aspects of both of these established research fields will be integral for future work applying an experimental assay to study bat orientation. Therefore we aimed to record baseline latency data with our CRBox setup. Our study is the first investigating wild migratory mammals with the help of an orientation arena.

## MATERIAL AND METHODS

### Study location and animals

Field work was conducted between 24 August and 10 September 2016 at Pape Bird Ringing Station (56°09' N 21°03' E, Rucava Municipality, Latvia) under permit Nr. 31/2016-E issued by the Latvian Nature Conservation Agency. Using a Heligoland funnel trap, we caught in total 64 adult

bats. We aged bats according to the closure of epiphyseal gaps of the phalanges; *P. nathusii*: 19 males, 16 females, *P. pygmaeus*: 12 males, 15 females, 2 individuals with undetermined sex. Bats were kept in wooden boxes in small groups of 3 to 4 individuals until tested.

**Test apparatus**

All components for construction of the orientation cage for bats, i.e. the CRBox, were purchased from hardware stores. As a measure of precaution, we only used non-magnetic materials to avoid interference with a putative magnetic sense of bats. The same rationale applied to the mechanism for remote release which is operated manually, as any electronics could also potentially interfere with magnetoreception due to electromagnetic noise (Engels et al. 2014). To ensure geomagnetic field intensity at the experimental site (50.8 µT, measured on 24 Aug 2016) was not altered inside the CRBox, we used an Apple iPhone 5 equipped with a 3D magnetometer (Asahi Kasei Microdevices AK8963, Tokyo, Japan; resolution: 0.15 µT/LSB, 16-bit) for control measurements. The CRBox consisted of two circular shaped elements, a lid made out of wood, including the mechanism for remote release, and the arena with eight openings for directional choice as the bottom (Fig. 1). The arena part consisted of a shallow funnel, so that a bat started a test below the level of the wall openings, i.e. a focal animal was placed in the centre of the funnel, at its lowest part, and was required to crawl for ~12 cm upwards to leave the arena through one of eight exits. In this way, animals would not face the openings immediately, and thus the effect of a spontaneous escape response is minimized (Fig. 1a). To construct the funnel, we used extruded polystyrol (XPS, Jackodur, Jackson Insulation). We applied synthetic leather as a coat for the arena because it is not slippery for crawling bats and it comes with a surface texture which can be cleaned easily between trials. The eight takeoff platforms were made out of wood and tilted downward 40° since an

inclined platform would facilitate takeoff for bats. Remote release of the focal bat is enabled through lifting the acclimatisation box (non-transparent PET) which was fixed to a stout cord running over a pulley. We attached a white stiff paper flag to the rod as a visual cue for the experimenter to stop pulling the chord when the acclimatisation box was lifted to the maximum. The lid of the CRBox extends beyond the arena (Fig. 1b). The brim serves to hinder the bat from seeing the night sky overhead and to curtail any emitted echolocation calls. If the bat is motivated to gather information from its surroundings, it is thus forced to takeoff. Finally, we used a stool to position the CRBox 1 m above ground to increase the likelihood for bats to takeoff (Fig. 1c). Using a spirit level, we adjusted the CRBox to the horizontal plane.

**Release procedure and orientation measures**

CRBox tests were conducted over the course of six nights on a meadow of rectangular shape (1,300 $m^2$) surrounded by trees, 100 m distant to the shoreline and only under calm wind conditions and clear sky. The CRBox was placed in the centre of the meadow with a minimum distance of 18 m to surrounding rows of trees. We used a bat detector (Pettersson D-100) tuned to the call frequency of the species under test (40 kHz for *P. nathusii* and 50 kHz for *P. pygmaeus* respectively) to control for any conspecific bat calls at the location of the CRBox. Bats are social animals and may orient towards or be repelled by echolocation or social calls emitted by conspecifics. This could affect exit latency, TOO and vanishing behaviour once a bat is airborne. We tested bats in the second half of the night, with the earliest release starting at 01:30 h and the latest at 05:30 h. We repeated acoustic checks for free flying wild bats between trials. Releases were paused when other bats were registered. Before any trial, the interior of the CRBox and takeoff platforms were cleaned with 70% ethanol to prevent animals from using olfactory cues left by previously tested bats. We

manually placed individuals into the acclimatisation box varying the angle for insertion randomly by 90° between tests. Bats were restricted from viewing spatial details of the environment prior to release. We used night vision goggles (BIG25 Safran Vectronix AG) for behavioural observations, i.e., comparison of TOO and subsequent flight. Departure flight bearings were recorded in eight sectors with a width of 45°, i.e., cardinal and ordinal compass directions after the bat had disappeared from view. Final bat vanishing directions could be observed up to a distance of approx. 20 to 50 m, depending on whether a focal bat flew into or above the canopy of trees surrounding the meadow. Departure bearings were noted from the position of the CRBox. The observer moved to this position when a bat was about to vanish from viewing range in heights above canopy. If a bat disappeared in the canopy, the observer memorised that spot by means of tree characteristics and recorded the bearing from the position of the CRBox. The error introduced by measuring all departure flight bearings from that single position is negligible considering the 45° wide sectors for recording of data. For analysis of bearing fidelity, we discarded trials in which we were not able to identify DFO due to rapid flight manoeuvres performed by these individuals, and thus could not calculate angular differences (*P. nathusii*, n = 13; *P. pygmaeus*, n = 5). However, we still used takeoff latency data from discarded trials as those were not compromised. Variances of bearing fidelity within and across animals can be evaluated based on angular differences between measures of directionality at different spatial scales, e.g., TOO and DFO. For intra- and inter-individual statistical comparison, the analysis of bearing fidelity requires normalisation of the initial orientation measure (here TOO) to a common direction. As a result, the second measure (DFO) can be visualised relative to the TOO of the bat. Measurement of takeoff latency started as soon as the acclimatisation box was lifted, i.e., when a bat was released inside of the arena, and stopped when the bat launched into air. Based on escape tests with several species of bats (Chase, 1981), we set a 3 minute cut-off time for cancelling a release trial. The experimenter at the other end of

the cord remained motionless to avoid any noise which could hinder bats from taking off or motivate them to change flight paths over the meadow. For *P. pygmaeus*, we measured takeoff latency during 22 trials; in five trials however, we could not record the takeoff precisely enough to include these measurements in the statistical analysis.

**Statistical analysis of orientation behaviour and takeoff latency**

To test for persistence between TOO and DFO, we normalised TOOs (e.g. N, NE, E, etc.) of all individuals for which we observed departure flights to a common direction (0° ahead) and determined the individual angular difference of respective departure flight bearings by subtraction. If TOO was the consequence of a spontaneous escape-response dependant on direction of insertion or the result of stress by the procedure, we would expect uniform random distribution of departure flight bearings irrespective of normalisation, since we randomly inserted animals to the test apparatus. We determined unimodal deviation from circular uniformity by applying Rayleigh's test (Batschelet 1981). For both species, we compared mean DFO with normalised TOO (0° ahead) by inspecting 95% confidence intervals, which is a way to evaluate whether mean DFO would encompass TOO (confidence interval test), i.e. if bearing fidelity can be assumed after a bat takes off. We further aimed to evaluate how actual vanishing directions of both species matched with the topography around the release site, e.g. whether they were aligned with the coastline, or if orientations resemble migratory orientations previously recorded via capture-recapture (ringing) and radio tracking (Pētersons 2004; Lindecke et al. 2015). At our field station, the coastline runs in a straight line from NNW (339.0°) to SSE (159.0°) in both directions. We calculated mean bearing and mean vector length from DFOs of both species while using Rayleigh's test to control for deviation from circular uniformity. However, we observed bats taking off and vanishing in

both, southerly and northerly directions, as well. Since orientations could eventually be bimodal, we analysed the data axially if $r_{axial} > r_{unimodal}$. Inspection of confidence intervals of bat data enabled us to compare it with coastline and site-specific migratory orientations in *P. nathusii*. All circular calculations were performed using Oriana 4.0 software (Kovach Computing Services, Pentraeth, UK).

We measured the time (seconds) a focal bat took to take off from the platforms after the acclimatisation box inside the CRBox got lifted. We compared takeoff latency among species by a Mann-Whitney U test (normality test failed, $P < 0.05$; SigmaPlot 11.0, Systat Software Inc., Chicago, IL, USA). We tested for differences between sexes using a Mann-Whitney U test for *P. nathusii* (normality test failed, $P < 0.05$) and an unpaired t-test for *P. pygmaeus* (normality test, $P = 0.164$).

## RESULTS

### Bearing fidelity

We measured movement directionality, i.e., TOO and corresponding DFO, in 45 released bats of two species *Pipistrellus nathusii* (n = 22), and *P. pygmaeus* (n = 23). Normalisation of individual TOOs to a common direction (0°), revealed a significantly unimodal distribution of departure flight bearings (Rayleigh test, *P. nathusii*: $r = 0.456$, $Z = 4.632$, $P = 0.008$; *P. pygmaeus*: $r = 0.605$, $Z = 8.427$, $P = 0.0001$; Fig. 2a, b). Further, in both species, mean vectors ($\mu$) of departure flights did not differ from takeoff bearings (95% confidence intervals for $\mu$, *P. nathusii*: 327.6° to 37.2° with $\mu = 2.4°$; *P. pygmaeus*: 354.7° to 51.0° with $\mu = 19.0°$; confidence interval tests for both species:

$P > 0.05$). However after takeoff, we observed occasionally bats circling above the CRBox before disappearing from the experimental area in a straight line, i.e. in DFO.

**Orientation in the environmental context**

Bearings of *P. nathusii* showed a bimodal distribution (Rayleigh test, $r_{axial} = 0.502$, $Z = 5.552$, $P = 0.003$; Fig. 2c) with a mean N–S vector which did not differ significantly from the coast line orientation at our study site (95% confidence intervals for *P. nathusii*: 184.8° to 153.4° with $\mu_{axial} = 169.1° – 349.1°$; confidence interval test, $P > 0.05$). *Pipistrellus pygmaeus* were oriented unimodally ($r_{unimodal} = 0.702$, $Z = 11.322$, $P = 2.53*e^{-6}$; Fig. 2d) with a mean vector in SSW (209°) direction.

**Takeoff latency**

Takeoff latency did not exceed 3 min in total for any of the tested bats (*P. nathusii*: n = 35, median: 55 s, range: 6–176 s; *P. pygmaeus*: n = 17, median: 17 s, range: 6–92 s; Fig. 3). We detected no difference in takeoff latencies between sexes (*P. nathusii*: U = 131.0, $P = 0.497$, $n_{males} = 19$, $n_{females} = 16$; *P. pygmaeus*: t = -0.183, $P = 0.858$, $n_{males} = 6$, $n_{females} = 9$, excluding two individuals with sex undetermined) and therefore we pooled data for interspecific comparison. *Pipistrellus nathusii* readily showed akinesia when carried and inserted into the CRBox, respectively. Consistent with our hypotheses, this may account for the significant difference in takeoff latency between the two species (Mann-Whitney U-Test, U = 154.0, $P = 0.004$).

## DISCUSSION

Close to a major migration corridor of bats at the coast of the Baltic Sea in Latvia, we compared the movement orientation of two congeneric bat species, one being a known long-distance migrant (*P. nathusii*) and the other with a putative migration behaviour (*P. pygmaeus*). Our observation of consistent southern heading directions of *P. pygmaeus* is in line with migratory behaviour of this species. Indeed, orientation and takeoff direction were similar to that of *P. nathusii*, a species with known long-distance migration across Europe. The seasonal occurrence of *P. pygmaeus* at our study site in conjunction with orientation towards the south in departing individuals identifies *P. pygmaeus* as a migratory species, at least for its north-eastern distribution range (Voigt et al. 2017, 2018). Using our novel experimental setup, called the circular release box (CRBox), we here showed (1) initial movements of bats are goal-oriented on the level of takeoff, that (2) orientations are biologically meaningful, and that (3) the principal methodology enables comparison of different measures between species. Therefore, we established that takeoff directions of bats are a suitable measure for developing an experimental paradigm for studies of mammal navigation and sensory ecology using bats as a model organism.

### Bat orientation relative to the environment

In both species, DFOs matched with the geographical orientation of the local coastline, i.e., the migratory corridor and direction, respectively (Pētersons 2004; Lindecke et al. 2015). For *P. nathusii*, we observed a bimodal orientation of movements in southern and northern direction, whereas *P. pygmaeus* flew in the direction in which bat migration is directed at PBRS in late summer (Lindecke et al. 2015). The observed orientations of *P. pygmaeus* would bring bats back

to the flyway above the coastal dunes or 50-100 m parallel to it. In a study by Ahlén et al. (2009) conducted at southern shores of Scandinavia, *P. pygmaeus* showed behaviour similar to *P. nathusii* and *N. noctula*, as well, as it was observed in high numbers at departure sites of these migratory species leaving for offshore flights. However, regarding our subsample of northerly flying *P. nathusii*, we hypothesise that these bats reversed orientation to find a day roost in the coastal forest or to forage instead of continuing migration along the dunes. This reverse orientation could have been caused due to the timing of our experiments, which only covered the second part of a night. Species-specific orientation responses can principally change during the activity period of bats (Mistry 1990). Interestingly, in migratory bird studies, similar observations of bimodal orientations have been made, especially at coastal sites (Sandberg et al. 1988; Åkesson et al. 1996). It has been hypothesised that such differences in orientation depend on the stage of migration with reverse movements along the migratory route occurring in individuals from populations which cover shorter distances (Muheim et al. 2017).

In roe deer it has recently been found that disturbed individuals directed their initial movements depending on the initial alignment of the body axis (Obleser et al. 2017). In contrast to roe deer individuals aligned on the east-west axis, individuals aligned along the north-south axis escaped not randomly, but toward north or south, respectively. These findings routed in the magnetic alignment hypothesis (Begall et al. 2013; Obleser et al. 2017) deserve consideration in future work based on takeoff as initial movement being measured in departing bats.

**Takeoff as measure**

Comparisons of takeoff with respect to subsequent flight demonstrated directional persistence between these movements, i.e., bearing fidelity exists between individual TOO and DFO in migratory *P. nathusii* and *P. pygmaeus*. Thus, we inferred that TOO as measured by the CRBox is

a suitable proxy to predict DFO after bats started flying. This observation supports our assumption that bats were able to acquire environmental information relevant for orientation and navigation, even in a relatively cue-deprived situation but with a multi-directional choice for takeoff, i.e., in the CRBox. Based on results gathered with a *resting assay* using a similar-sized setup in the laboratory (Wang et al., 2007), we speculate that the Earth's magnetic field might have played a functional role for our bats. Yet again, it is too early to draw further inferences about the role of magnetoreception in bat orientation on the level of takeoff. For example, this would require an experimental setup based on magnetic coils where the geomagnetic-field around a focal bat could be precisely manipulated. However, at the perimeter of the CRBox, echolocation would not have provided useful long-distance information about landmarks at the moment of release, and view of the nightly sky with stars, Milky Way or moon, was inhibited by the design of the CRBox as well (Fig. 1). However, environmental olfactory cues or distant low-frequency sounds, e.g. emitted by the Baltic Sea, might have influenced TOO of our bats as well (Childs & Buchler, 1981; Gagliardo, 2013). Previously, bat orientation studies measuring initial flight orientations visually were limited to sampling under twilight conditions at dusk and dawn (Davis, 1966; Buchler & Childs, 1981, 1982; Serra-Cobo, et al. 2000). In our study, application of night-vision technique for behavioural observations proved to be a solution to avoid effects of environmental light to which bats might be sensitive (Chase, 1981; Buchler & Childs, 1982; Holland et al. 2010; Greif, et al. 2014). However, we missed some flights due to rapid movements of animals even using advanced optical equipment.

We recorded sufficient baseline data about takeoff latencies for the two pipistrelle species and found differences, with *P. nathusii* performing significantly slower than *P. pygmaeus*. Compared to *P. pygmaeus*, *P. nathusii* readily shows a state of motionless inactivity, so-called akinesis, when being handled. We assume this behaviour of *P. nathusii* in response to handling was delaying takeoff. Yet, with respect to bearing fidelity, *P. nathusii*, like *P.pygmaeus*, qualifies as an

appropriate model species for orientation tests with relatively short release trials (< 3 min) which are practicable for testing multiple independent subjects over a short period. Previous research using crawling bats in Y-maze tests suggested a true escape response would take only two to three seconds for active bats in similar settings (Chase, 1981; Mistry 1990). Here observed latencies suggest that our bats did not escape spontaneously but took off after a delay putatively serving orientation in the confined space of the CRBox.

In principle, using an arena-based approach to elucidate preferred bat orientations adds to the methodological repertoire for the study of bat species. Eventually, this could be useful for identification of migration routes of other bats across their ranges. But primarily, testing directionality of takeoff represents a novel option for addressing a multitude of questions in bat physiology and sensory ecology where circular orientation responses have rarely been recorded before. We acknowledge that for certain bat families the current CRBox is eventually unsuitable, e.g. New World leaf-nosed bats (Phyllostomidae; but see Chase, 1981 & 1983 and Gröger & Wiegrebe, 2006) and Old world Horseshoe bats (Rhinolophoidae) which commonly do not use quadrupedal locomotion, i.e. crawling. However, for families like Vespertilionidae, Miniopteridae or Molossidae, which readily crawl on the ground or in crevices, TOO measures have high potential for orientation studies, as the principle CRBox design allows measurement of a natural behaviour in these species. Yet, for future works the CRBox should be improved to gain higher resolution of orientation measures, e.g. by offering more exits. This would allow for more sophisticated test procedures enabling statistical comparison between study species or experimental groups (Batschelet 1981).

## Conclusions and open questions

Our results suggest *P. pygmaeus* from northern Europe behaves similar to long-distance migratory *P. nathusii* during times of summer migration at the Baltic Sea coast (Lindecke et al. 2015, and this study). The observed orientations would lead bats southwards and back to the flyway along the dunes of the shoreline. Based on our findings combined with previous results about population genetics, phenological occurrences and flight behaviour at sea sides, we argue that *P. pygmaeus* can be considered a true migratory species, at least in northern parts of its distribution range (Ahlén et al. 2009, Sztencel-Jabłonka & Bogdanowicz 2012; Rydell et al. 2014, Voigt et al. 2017, 2018). Quantitative studies based on tagged individuals are now needed to evaluate how regular *P. pygmaeus* migrate over longer distances and where individuals of northern populations are overwintering. Still, it remains unresolved which environmental cues contribute to the compass system bats and other mammals use for migration. Pipistrelle species appear to be a suitable future model to address this question in detail. With an easy-to-build experimental arena setup, we established a method to study initial orientation behaviour of bats, using their natural takeoff behaviour as a proxy for directional choices. Future work is necessary to understand which environmental cues enable bats to orient in the small, confined space of a test arena. We speculate one of the most important cues to be the geomagnetic field (Holland et al. 2006; Wang et al. 2007).

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**Author contributions:** O.L. conceived the study, designed and constructed the behavioural test, analysed the data and led the manuscript writing; O.L. and A.E. performed experiments; C.C.V. and G.P. supported fieldwork; C.C.V. and G.P supervised the students; O.L., R.A.H. and C.C.V. interpreted the data and wrote the manuscript.

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## Figure legends

**Figure 1.** The circular release box for bats. (a) Cross-section view, with dimensions (in mm) adapted for small bat species of < 80 mm body length. 1 = pulley for remote release, 2 = acclimatisation box with rod and white signal flag, 3 = funnel, 4 = takeoff platform. (b) Top view of the CRBox with takeoff platforms oriented in cardinal and ordinal directions. The roof board is shown transparent. (c) CRBox setup photographed through night vision goggles.

**Figure 2.** Orientation behaviour of pipistrelle bats. Circular graphs on the left show normalised departure flight bearings (relative to individual takeoff orientation) of *P. nathusii* (a) and *P. pygmaeus* (b) which were significantly oriented unimodally in both species (*P. nathusii*, n = 22; *P. pygmaeus*, n = 23). Mean vectors ($\mu$) of normalised departure flights were well within the orientation of takeoff (*P. nathusii*: $\mu$ = 2.4°; *P. pygmaeus*: $\mu$ = 19.0°). Directions of arrows depict the orientation of $\mu$ and the lengths reflect the *r* proportional to the radius of the circle. The 95% confidence intervals are indicated above the mean vector. The two inner circles mark the 5% (dotted) and the 1% significance border. Numbers on gridlines indicate sample sizes covered by the wedges. Circular graphs on the right show departure flight orientations in an environmental context with the Baltic Sea in the west. Vanishings of *P. nathusii* (c) were bimodal with a mean

vector similar to the coastal orientation while the mean vector of *P. pygmaeus* (d) was oriented SSW.

**Figure 3. Takeoff latency of bats.** Boxplots depict median and mean latency (dashed); outliers are indicated by points not covered by the whiskers (*P. nathusii*: n = 35; *P. pygmaeus*: n = 17). The inlet photograph shows a *P. pygmaeus* crawling onto a platform for takeoff. Takeoff latency in both species differed significantly ($P = 0.004$).